nucleotide sequences, if present in the sample. The probes also ligate to one another to form a ligation product sequence containing the target specific portions connected together with the ligation product sequences for each set being distinguishable from other nucleic acids in the ligase chain reaction mixture. The oligonucleotide probe sets may hybridize to nucleotide sequences in the sample other than their respective target nucleotide sequences but do not ligate together due to a presence of one or more mismatches and individually separate during the denaturation treatment. The presence of ligation product sequences produced as a result of the target nucleotide sequence being present in the sample are then detected.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-C show a sequence comparison of *Thermus* DNA ligases. Figure 1A illustrates the evolutionary tree for *Thermus* DNA ligases. Figure 1B is a regional sequence alignment of nine *Thermus* ligases. The aa (i.e. amino acid) sequence of T. scot is retrieved from Genebank by accession number 1085749. The adenylation motif KXDG is underlined and the adenylation site is marked by *. The numbering of aa is based on *Tsp.* AK16D ligase. Figure 1C is a complete amino acid sequence of *Tsp.* AK16D ligase. The adenylation motif KXDG is underlined and the adenylation site ^{118}K is shown with a (*) above the residue. The complete sequence of *Tsp.* AK16D ligase gene and partial sequences of six other *Thermus* ligase genes have been deposited with GenBank under accession No. AF092862 for *Tsp.* AK16D, AF092863 for *Thermus aquaticus* YT-1, AF092864 for *Thermus flavus*, AF092865 for *Thermus filiformis* Tok4A2, AF092866 for *Thermus filiformis* Tok6A1, AF092867 for *Tsp.* Vil3, and AF092868 for *Tsp.* SM32.

Figure 2 shows an SDS-PAGE analysis of *Tsp.* AK16D ligase protein. Lane 1, molecular weight markers; Lane 2, uninduced cell lysate; Lane 3, induced cell lysate; Lane 4, supernatant after heating at 70°C; Lane 5, fraction eluted from Hitrap blue column. The SDS-polyacrylamide gel was 0.1% SDS-7.5% polyacrylamide and was stained with Coomassie brilliant blue after electrophoresis. The arrow points to the location of *Tsp.* AK16D ligase.

Figures 3A-C show the effects of salt, pH, and NAD^+ on ligation

activity. *Tsp.* AK16D ligase: closed squares; *Tth* ligase: open squares. Figure 3A reveals the pH effect. Reactions were performed in 20 μl mixture containing 200 nM nicked duplex substrate, 12.5 pM *Tth* ligase or *Tsp.* AK16D ligase, 20 mM Tris-HCl (pH values were determined at room temperature), 10 mM $MgCl_2$, 100 mM KCl, 10 mM DTT, 1 mM NAD^+ and 20 mg/ml BSA at 65°C for 10 min. Figure 3B shows the salt effect. Reactions were performed in 20 μl mixture containing 200 nM nicked duplex substrate, 12.5 pM *Tth* ligase or *Tsp.* AK16D ligase, 20 mM Tris-HCl, pH 8.5 (at room temperature) for *Tth* ligase, pH 8.0 for *Tsp.* AK16D ligase, 10 mM $MgCl_2$, indicated amount of KCl, 10 mM DTT, 1 mM NAD^+ and 20 mg/ml BSA at 65°C for 10 min. Figure 3C shows the NAD^+ effect. *Tth* ligation reactions were performed in 20 μl mixture containing 200 nM nicked duplex substrate, 12.5 pM *Tth* ligase and indicated concentration of NAD^+, 20 mM Tris-HCl, pH 8.5, 5 mM $MgCl_2$, 100 mM KCl, 10 mM DTT, 1 mM NAD^+ and 20 mg/ml BSA at 65°C for 10 min. *Tsp.* AK16D ligation reaction were performed in 20 μl mixture containing 200 nM nicked duplex substrate, 12.5 pM *Tth* ligase and indicated concentration of NAD^+, 20 mM Tris-HCl, pH 8.5, 5 mM $MgCl_2$, 50 mM KCl, 10 mM DTT, 1 mM NAD^+ and 20 mg/ml BSA at 65°C for 10 min.

Figures 4A-B show the divalent cation dependence of *Tsp.* AK16D (stripped bars) and *Tth* (filled bars) ligase activity. Reaction mixtures containing (20 μl) 20 nM nicked duplex substrate, 0.5 nM *Tth* ligase or 1 nM *Tsp.* AK16D ligase and 5 mM of indicated divalent cation in the reaction buffers as specified in Figure 3C were incubated at 65°C for 10 min. Figure 4A shows the ligation reactions with different divalent ions as the metal cofactor. Figure 4B shows the chromatogram of a representative GeneScan gel illustrating ligation product and DNA adenylate intermediate. (-): negative control reactions in which ligase was omitted. Co^{2+} may have caused precipitation of DNA substrate which resulted in disappearance of the unreacted substrate.

Figures 5A-B shows the time course of *Tth* (Figure 5A) and *Tsp.* AK16D (Figure 5B) ligase activity in the presence of Mg^{2+} (open squares) or Mn^{2+} (closed squares). Reactions were performed in 100 μl mixture containing 20 nM nicked duplex substrate, 0.5 nM *Tth* ligase or 1 nM *Tsp.* AK16D ligase and 5 mM

Mg^{2+} or Mn^{2+} in the reaction buffers as specified in Figure 3C at 65°C. Aliquots (5 µl) were removed at the indicated time and reactions stopped by adding equal volumes of stop solution.

Figures 6A-B show the divalent cation concentration dependence of *Tth* (Figure 6A) and *Tsp.* AK16D (Figure 6B) ligase activity. Mg^{2+}(open squares); Mn^{2+} (closed squares). Reactions were performed in 20 µl mixture containing 20 nM nicked duplex substrate, 0.5 nM *Tth* ligase or 1 nM *Tsp.* AK16D ligase and indicated concentration of Mg^{2+} or Mn^{2+} in the reaction buffers as specified in Figure 4C at 65°C for 2 min.

Figures 7A-B show the ligation of gapped and inserted substrates. Figure 7A shows the formation of ligated product with gapped and inserted substrates. Reactions were performed in a 20 µl mixture containing 12.5 nM nicked duplex substrate, 1.25 pM *Tth* ligase or 12.5 nM *Tsp.* AK16D ligase in the reaction buffer at 65°C for 4 hours. Figure 7B shows the proposed reaction path leads to ligation of 1 nt (i.e. nucleotides) inserted substrate.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a high fidelity thermostable ligase enzyme. This enzyme has the amino acid sequence of SEQ. ID. No. 1 as follows:

MTLEEARRRVNELRDLIRYHNYLYYVLDAPEISDAEYDRLLRELKELEERFPELKSP
DSPTEQVGARPLEATFRPVRHPTRMYSLDNAFSLDEVRAFEERIERALGRKGPFLYT
VERKVDGLSVNLYYEEGILVFGATRGDGETGEEVTQNLLTIPTIPRRLTGVPDRLEV
RGEVYMPIEAFLRLNQELEEAGERIFKNPRNAAAGSLRQKDPRVTARRGLRATFYAL
GLGLEETGLKSQHDLLLWLRERGFPVEHGFTRALGAEGVEEVYQAWLKERRKLPFEA
DGVVVKLDDLALWRELGYTARTPRFALAYKFPAEEKETRLLSVAFQVGRTGRITPVG
VLEPVFIEGSEVSRVTLHNESFIEELDVRIGDWVLVHKAGGVIPEVLRVLKERRTGE
EKPIIWPENCPECGHALIKEGKVHRCPNPLCPAKRFEAIRHYASRKAMDIQGLGEKL
IEKLLEKGLVRDVADLYRLKKEDLVNLERMGEKSAENLLRQIEESKGRGLERLLYAL
GLPGVGEVLARNLALRFGHMDRLLEAGLEDLLEVEGVGELTARAILNTLKDPEFRDL
VRRLKEAGVEMEAKEREGEALKGLTFVITGELSRPREEVKALLRRLGAKVTDSVSRK
TSFLVVGENPGSKLEKARALGVPTLSEEELYRLIEERTGKDPRALTA